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DISTORTED EQUILIBRIUM CONFIGURATIONS OF A PERFECT COMPRESSIBLE FLUID MASS

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The problem of determining the distortion of a perfect, compressible fluid mass subject to the action of given forces is one which has apparently not been treated with a view to obtaining solutions of maximum generality. It is proposed to consider a general formulation of the problem for the cases in which the forces are tidal or rotational and to outline the steps by which a formal solution can be obtained.

In prospect, it should be noted that the problem has been treated in detail for polytropes,[1] i.e., configurations whose pressure-density relation is of the form

$$P = K\rho^{1+1/n} + D,$$

where P is the pressure at any point, ρ is the density at that point, n is a parameter called the "polytropic index" and K and D are constants. The methods used for polytropes can be extended to the treatment of configurations having arbitrary pressure-density relations and are, in fact, the methods to be used.

First to be considered is the problem of rotation. Let a perfect compressible fluid mass be at rest in an otherwise empty space. In static gravitational equilibrium the mass will assume a spherical form. If, however, the fluid mass (a star) partakes of a slow rotation about some fixed axis through its center, then the configuration will no longer be spherical; the "star" will have suffered a small "perturbation" from the static gravitational equilibrium which would prevail were it not rotating.

The equations of the problem are

$$P = P(\rho), \tag{1}$$

$$\frac{1}{\rho}\cdot\frac{\partial P}{\partial r} = \frac{\partial V}{\partial r} + r(1-\mu^2)\omega^2, \tag{2}$$

and

$$\frac{1}{\rho}\cdot\frac{\partial P}{\partial \mu} = \frac{\partial V}{\partial \mu} - r^2\mu\omega^2. \tag{3}$$

The first relates the pressure P at any point to the density ρ at that point by some *arbitrary* functional relationship. The second and third are the hydrodynamical equations, in which r is the radius vector from the center of the configuration, μ the cosine of the angle φ from the axis of rotation, ω the (constant) angular velocity of rotation and V the gravitational potential function satisfying Poisson's equation

$$\frac{1}{r^2}\left\{\frac{\partial}{\partial r}\left(r^2\frac{\partial V}{\partial r}\right) + \frac{\partial}{\partial \mu}\left((1-\mu^2)\frac{\partial V}{\partial \mu}\right)\right\} = -4\pi G\rho. \tag{4}$$

These equations can be reduced to a set of dimensionless equations by the transformations

$$\begin{aligned} r &= \alpha\xi, \\ \rho &= \rho_c\zeta, \\ P &= (4\pi G\rho_c^2\alpha^2)\eta = P_c\eta, \\ V &= (4\pi G\rho_c\alpha^2)\chi, \end{aligned} \tag{5}$$

where P_c and ρ_c are the central pressure and central density, respectively. Then, letting

$$\epsilon = \frac{\omega^2}{4\pi G\rho_c}, \tag{6}$$

the equations of the problem become

$$\eta = \eta(\zeta), \tag{7}$$

$$\frac{1}{\zeta}\frac{\partial \eta}{\partial \xi} = \frac{\partial \chi}{\partial \xi} + \epsilon\xi(1-\mu^2), \tag{8}$$

$$\frac{1}{\zeta}\frac{\partial \eta}{\partial \mu} = \frac{\partial \chi}{\partial \mu} - \epsilon\xi^2\mu, \tag{9}$$

and

$$\frac{1}{\xi^2}\left\{\frac{\partial}{\partial \xi}\left(\xi^2\frac{\partial \chi}{\partial \xi}\right) + \frac{\partial}{\partial \mu}\left((1-\mu^2)\frac{\partial \chi}{\partial \mu}\right)\right\} = -\zeta. \tag{10}$$

It is now assumed that ζ, η and χ can be expressed as power series in the parameter ϵ, having the respective forms

$$\zeta(\xi, \mu; \epsilon) = \zeta^{(0)}(\xi) + \epsilon[\zeta_0^{(1)}(\xi) + \sum_{j=1}^{\infty} \zeta_j^{(1)}(\xi)P_j(\mu)] + \ldots, \quad (11)$$

$$\eta(\xi, \mu; \epsilon) = \eta^{(0)}(\xi) + \epsilon[\eta_0^{(1)}(\xi) + \sum_{j=1}^{\infty} \eta_j^{(1)}(\xi)P_j(\mu)] + \ldots, \quad (12)$$

and

$$\chi(\xi, \mu; \epsilon) = \chi^{(0)}(\xi) + \epsilon[\chi_0^{(1)}(\xi) + \sum_{j=1}^{\infty} \chi_j^{(1)}(\xi)P_j(\mu)] + \ldots, \quad (13)$$

where $P_j(\mu)$ is the Legendre polynomial of index j, and $\zeta^{(0)}$, $\eta^{(0)}$ and $\chi^{(0)}$ describe an undistorted equilibrium configuration, or in other words, are solutions of the equations

$$\eta^{(0)} = \eta(\zeta^{(0)}),$$

$$\frac{1}{\zeta^{(0)}} \frac{d\eta^{(0)}}{d\xi} = \frac{d\chi^{(0)}}{d\xi}, \quad (14)$$

$$\frac{1}{\xi^2} \left\{ \frac{d}{d\xi} \left(\xi^2 \frac{d\chi^{(0)}}{d\xi} \right) \right\} = -\zeta^{(0)}.$$

Straightforward substitution enables one to perform a separation of variables and leads to a set of differential equations in ξ alone. Their solutions, together with equation (7) and the set of Legendre polynomials, yield a complete formal solution. In order that it be an actual solution, however, it must satisfy certain appropriate boundary conditions.

In the first place, the separate gravitational potential functions for the regions inside and outside the configuration must be joined smoothly at the outer boundary of the star, i.e., the functions and their first derivatives must be equal on this surface. This can be shown to imply that

$$\eta_j^{(1)}(\xi) \equiv 0, \ (j \neq 0, 2) \quad (15)$$

$$\lim_{\xi \to \xi_0} \left\{ 3 \left(\frac{\eta_2^{(1)}}{\zeta^{(0)}} \right) + \xi \left[\frac{d}{d\xi} \left(\frac{\eta_2^{(1)}}{\zeta^{(0)}} \right) \right] \right\} = -(5/3)\xi_0^2, \quad (16)$$

where $\xi = \xi_0$ is the outer boundary of the original, unperturbed configuration. Further, it can be shown to follow from (7) and (15) that

$$\zeta_j^{(1)}(\xi) \equiv 0. \quad (j \neq 0, 2) \quad (17)$$

With these results, the differential equations to be solved are found to be

$$\frac{1}{\xi^2}\left\{\frac{d}{d\xi}\left(\xi^2\frac{d}{d\xi}\left[\frac{\eta_0^{(1)}}{\zeta^{(0)}}\right]\right)\right\} = 2 - \eta_0^{(1)}\left(\frac{(d\zeta^{(0)}/d\xi)}{(d\eta^{(0)}/d\xi)}\right), \tag{18}$$

and

$$\frac{1}{\xi^2}\left\{\frac{d}{d\xi}\left[\xi^2\frac{d}{d\xi}\left(\frac{\eta_2^{(1)}}{\zeta^{(0)}}\right)\right] - 6\left(\frac{\eta_2^{(1)}}{\zeta^{(0)}}\right)\right\} = -\eta_2^{(1)}\left(\frac{(d\zeta^{(0)}/d\xi)}{(d\eta^{(0)}/d\xi)}\right). \tag{19}$$

After the solutions of equations (18) and (19) have been found, the functions $\zeta_0^{(1)}$ and $\zeta_2^{(1)}$ can be obtained by means of the relations

$$\zeta_0^{(1)} = \left(\frac{(d\zeta^{(0)}/d\xi)}{(d\eta^{(0)}/d\xi)}\right)\eta_0^{(1)}, \tag{20}$$

$$\zeta_2^{(1)} = \left(\frac{(d\zeta^{(0)}/d\xi)}{(d\eta^{(0)}/d\xi)}\right)\eta_2^{(1)}. \tag{21}$$

It should be noted that a derivation of these last four equations depends on the assumption that a relation such as (7) exists; its exact form is irrelevant but its existence is necessary.

Now, equations (18) and (19) are second order ordinary differential equations and therefore their general solutions contain two constants which are to be fixed by imposing on them appropriate initial conditions. If, instead of comparing rotating and non-rotating configurations of the same total mass, as originally proposed, one chooses to compare configurations of equal central density[2] (and pressure), one then has the initial conditions

$$\eta_{\xi=0} = \eta^{(0)}_{\xi=0} = 1, \left(\frac{d\eta}{d\xi}\right)_{\xi=0} = 0, \tag{22}$$

whence it follows that

$$\eta_0^{(1)}(\xi = 0) = 0, \left(\frac{d\eta_0^{(1)}}{d\xi}\right)_{\xi=0} = 0,$$

$$\eta_2^{(1)}(\xi = 0) = 0, \left(\frac{d\eta_2^{(1)}}{d\xi}\right)_{\xi=0} = 0. \tag{23}$$

By these conditions $\eta_0^{(1)}$ is uniquely determined. Not so for $\eta_2^{(1)}$, however; for, since equation (19) is linear and homogeneous in $\eta_2^{(1)}$, solutions satisfying (23) are arbitrary to the extent of an arbitrary constant factor. Thus, if $\eta_2^{(1)*}$ is a solution, so also is $a_2^{(1)}\eta_2^{(1)*}$, where $a_2^{(1)}$ is any constant. Hence, for definiteness, one could choose $\eta_2^{(1)*}$ to be approximately pro-

portional to ξ^2 near $\xi = 0$ and in this case the constant $a_2^{(1)}$ would be fixed by the condition (16) which would become

$$a_2^{(1)}\left[\lim_{\xi \to \xi_0}\left\{3\left(\frac{\eta_2^{(1)*}}{\zeta^{(0)}}\right)+\xi\left[\frac{d}{d\xi}\left(\frac{\eta_2^{(1)*}}{\zeta^{(0)}}\right)\right]\right\}\right] = -(5/3)\xi_0^2. \quad (24)$$

The constant $a_2^{(1)}$ will be found to have a general analog in the tidal problem and is a parameter of considerable importance, the oblateness, for example, being expressible in terms of it.

In principle, therefore, the problem of the distortion of a compressible fluid configuration by rotation has been solved by a perturbation method—accurate to the first order in ϵ. The same general procedure is applicable to the treatment of the distortion of a compressible fluid configuration by the tidal force of an inverse square field.

Let a configuration be subject to the tidal action of a particle of mass M'' at a distance R from the center of the configuration and in the direction $\varphi = 0$ (or $\mu = 1$). Letting

$$R = \alpha\tau \quad (25)$$

and

$$\chi_0'' = \frac{M''}{4\pi\rho_c\alpha\tau}, \quad (26)$$

it is found that the functions ζ, η and χ have the series expansions

$$\zeta(\xi, \mu; \chi_0'') = \zeta^{(0)}(\xi) + \chi_0''\left[\sum_{j=2}^{4}\zeta_j^{(1)}(\xi)P_j(\mu)\right] + \ldots, \quad (27)$$

$$\eta(\xi, \mu; \chi_0'') = \eta^{(0)}(\xi) + \chi_0''\left[\sum_{j=2}^{4}\eta_j^{(1)}(\xi)P_j(\mu)\right] + \ldots, \quad (28)$$

$$\chi(\xi, \mu; \chi_0'') = \chi^{(0)}(\xi) + \chi_0''\left[\sum_{j=2}^{4}\chi_j^{(1)}(\xi)P_j(\mu)\right] + \ldots, \quad (29)$$

where $\zeta^{(0)}$, $\eta^{(0)}$ and $\chi^{(0)}$ are defined as before and the $\eta_j^{(1)}$ and $\zeta_j^{(1)}$ are found from the equations

$$\frac{1}{\xi^2}\left\{\frac{d}{d\xi}\left(\xi^2\frac{d}{d\xi}\left[\frac{\eta_j^{(1)}}{\zeta^{(0)}}\right]\right) - j(j+1)\left(\frac{\eta_j^{(1)}}{\zeta^{(0)}}\right)\right\} = -\eta_j^{(1)}\left(\frac{(d\zeta^{(0)}/d\xi)}{(d\eta^{(0)}/d\xi)}\right), \quad (j = 2, 3, 4) \quad (30)$$

and

$$\zeta_j^{(1)} = \eta_j^{(1)}\left(\frac{(d\zeta^{(0)}/d\xi)}{(d\eta^{(0)}/d\xi)}\right). \quad (j = 2, 3, 4) \quad (31)$$

The boundary conditions to be satisfied by the $\eta_j^{(1)}$ are

$$\eta_j^{(1)}(\xi = 0) = 0, \left(\frac{d\eta_j^{(1)}}{d\xi}\right)_{\xi=0} = 0, \ (j = 2, 3, 4) \tag{32}$$

$$\lim_{\xi \to \xi_0} \left\{ (j+1)\left(\frac{\eta_j^{(1)}}{\zeta^{(0)}}\right) + \xi\left[\frac{d}{d\xi}\left(\frac{\eta_j^{(1)}}{\zeta^{(0)}}\right)\right]\right\} = (2j+1)\left(\frac{\xi_0}{\tau}\right)^j.$$

$$(j = 2, 3, 4) \tag{33}$$

As before, one can let $\eta_j^{(1)} = a_j^{(1)}\eta_j^{(1)*}$, where $\eta_j^{(1)*} \to \xi^2$ as $\xi \to 0$, whence the $a_j^{(1)}$ are fixed by (33). These $a_j^{(1)}$ are of considerable importance as parameters relating to a description of the distorted configuration.[3]

The double-star problem is essentially a combination of the rotational and tidal problems. In this instance, the density, for example, can be expanded as

$$\zeta(\xi, \mu, \theta; \chi_0'', \epsilon) = \zeta^{(0,0)}(\xi) + \chi_0'' \sum_{j=0}^{\infty} \zeta^{(1,j,0)}(\xi)P_j(\mu) + \epsilon \sum_{j=0}^{\infty} \zeta^{(0,j,1)}(\xi)S_j(\mu, \theta) + \ldots, \tag{34}$$

where $\zeta^{(0,0)}$ corresponds to the $\zeta^{(0)}$ used previously and $S_j(\mu, \theta)$ is the arbitrary surface harmonic of index j. Substitution of these series into the equations of the problem give equations entirely analogous to those obtained in the previous two problems and will not be written out here.

In sum, then, the method applies generally and the approximation is accurate to the first order in an appropriate (constant) perturbation parameter or parameters. The generality derives from the fact that no specific pressure-density relation need be assumed; only the existence of such a relation is required. Indeed, a statement of a particular relation is made quite unnecessary. This enables one to obtain results from any known static equilibrium configuration. It is proposed to give numerical results and details of the argument in a subsequent publication.

It is a pleasure to acknowledge my indebtedness to Dr. Chandrasekhar for his guidance and stimulation.

[1] Chandrasekhar, S., *M. N.*, **93**, "The Equilibrium of Distorted Polytropes:" I, The Rotational Problem, p. 390; II, The Tidal Problem, p. 449; III, The Double-Star Problem, p. 462; IV, The Rotational and Tidal Distortions as Functions of the Density Distribution, p. 540.

[2] Comparison of rotating and non-rotating configurations having the same central density is much simpler than comparing configurations of equal total mass; a direct solution of the latter problem leads to many difficulties. However, given a solution of either problem, a solution of the other can be obtained from it.

[3] The quantity $\Delta_j^{(1)} = a_j^{(1)}\left(\frac{\xi_0}{\tau}\right)^{-j}\left[\lim_{\xi \to \xi_0}\left(\frac{\eta_j^{(1)*}}{\zeta^{(0)}}\right)\right]$ corresponds to the Δ's defined in Chandrasekhar's papers (see first reference above).

SOME LETHAL EFFECTS OF ULTRA-VIOLET RADIATION ON FUNGUS SPORES

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Smith[1] has thoroughly reviewed the literature up to 1935 on the effects of radiation on the fungi. Discussion in relation to the work here reported has been limited to recent papers dealing with yeast and with the filamentous fungi.

Several studies have been made with the object of determining the shape of the survival curve in an attempt to disclose the mechanism by which radiation effects the killing of single cells. Smith[2] used the full spectrum of the quartz mercury vapor lamp and irradiated spores of *Fusarium*. She studied not only the shape of the survival curve at a constant temperature, but also the variation in the shape of this curve with increasing temperature, so that temperature became a contributing lethal factor. She obtained sigmoid survival curves at lower temperatures and logarithmic curves when the temperature was increased. Oster[3] also studied the effect of temperature and calculated the coefficients for killing of yeast by monochromatic ultra-violet radiation.

Oster constructed a curve relating wave-length to the efficiency of killing of yeast. Hollaender and Emmons[4] worked with *Trichophyton mentagrophytes* and have recently established a similar curve for this organism. Both of these investigations indicated that a maximum killing efficiency is attained at wave-length 2650 A. These results have recently been confirmed for *Ustilago zeae*.[5]

Various investigators have published data indicating that the survival curve of yeast and the fungi is sigmoid in form.[3-6]

The relation between the age of the fungus and the amount of energy required to kill cells was studied for yeast by Oster[3] and by Schreiber.[7] Whereas Oster observed that increased amounts of energy were required to kill older cells, Schreiber noted little effect of age.

In our studies the method of irradiation employed was essentially that used by Duggar and Hollaender.[8] The source of radiation was an 85-watt type H-4 mercury vapor lamp made by the General Electric Company. The outer glass casing of the lamp was removed. Monochromatic radiation was obtained by means of a Bausch and Lomb quartz monochromator, kindly loaned for this work by the Department of Physics of the University of Wisconsin. Throughout this work, monochromatic radiation of wave-length 2650 A was employed. A thermopile in conjunction with a Leeds and Northrup high sensitivity galvanometer was used for determin-

ing the amount of radiation absorbed by the spore suspension in the exposure cell. The amount of energy absorbed was determined by the standard photochemical procedure. Irradiation was carried out at a temperature of 25°C.

Three species of fungi were used in this work. A culture of *Rhizopus suinus* Niels., originally obtained from Utrecht and maintained in culture here for several years, was used because of its rapid growth, repeatable behavior in culture and prolific spore production, the spores being only moderately pigmented. The minus strain of *Mucor dispersus* Hagem, obtained through the kindness of Prof. A. F. Blakeslee, was used because its spores were free of any pigmentation. Both *R. suinus* and *M. dispersus* produce multinucleate spores. *Aspergillus melleus* Yukawa, obtained from the collection of Aspergilli maintained by the Department of Agricultural Bacteriology of this university, was also used for the reason that the spores were pigmented and available in large quantities. Thom and Church[9] state that, according to Dangeard, the spores of many of the Aspergilli are uninucleate. We have assumed the production of uninucleate spores by this species of *Aspergillus*.

Stock cultures of *Rhizopus* and of *Aspergillus* were grown on a liquid medium and were incubated at 28°C. Since the mycelium of the *Mucor* tended to grow submerged in liquid media, it was grown on potato dextrose agar in Erlenmeyer flasks. Except for this procedure, the method of treatment was identical with that used for the other two fungi.

Spore suspensions for irradiation were prepared in the following manner: The liquid from a flask stock culture was poured off and the mycelium was rinsed with a few cc. of physiological salt solution[8] diluted to half strength. After two rinsings under sterile conditions, fresh salt solution was added. The flask was stoppered with a sterile cork and shaken thoroughly. The resulting suspension was then filtered through sterile absorbent cotton to remove mycelial fragments and clumped spores. The density of spores in the suspension to be irradiated was determined by means of a hemocytometer. The suspensions to be irradiated were of such concentration that complete absorption of the incident beam of radiation did not occur. This procedure was adopted in order that the spores in the beam of radiation would be "saturated" with lethally effective quanta. Fungous spores of these species are far more resistant to ultra-violet radiation than are vegetative bacterial and yeast cells, and it is therefore necessary to use suspensions of low spore concentration in order to effect high killing in a reasonable time. Such a procedure also minimizes effects other than those of radiation.

In making intensity measurements, the difference between the deflection of the galvanometer when the theromopile was placed behind the cell filled with spore suspension and that obtained behind the cell filled with salt solution was used as a measure of the light absorbed by the fungous spores

after making suitable corrections for reflection of light by the exposure cell.

Since a stirring mechanism in the beam of incident radiation would absorb and scatter considerable quantities of radiation, no mechanical stirrer was used to agitate the spore suspension in the exposure cell. Frequent agitation of the spore suspension was necessary to prevent sedimentation of spores and was effected by means of a pipette during the course of exposure.

After a suitable time interval, $^1/_{10}$-cc. samples of spore suspension were removed from the exposure cell. These were diluted with a potato dextrose solution consisting of the broth of 200 g. of potatoes, 20 g. dextrose and 1 g. agar in 1000 cc. of distilled water. This quantity of agar was just sufficient to cause gelation of the medium so that sedimentation of the spores in the hanging drop was prevented. Ten volumes of this nutrient were added to one volume of spore suspension. This medium was used directly in making hanging drop cells, according to the standard procedure.

Preliminary experiments, carried out in order to ascertain the amount of time required for the process of germination to become complete, showed that a period of 15 to 20 hours was adequate, the time being dependent upon the dosage of radiation given the spore suspension. This was true under the experimental conditions employed for each of the three species studied.

Hanging drop cells were incubated at 28°C. for 15 to 20 hours and counts were then made of the number of germinating spores as well as of the total number of spores. A minimum of 200 spores was counted for each hanging drop culture and such cultures made in duplicate, or in greater replication, were prepared for each type of exposure. The average value of the percentage germination was determined in each case.

Control suspensions of spores were made up and were treated identically with exposed suspensions in every way, except that they were not exposed to radiation. Exposed suspensions were irradiated for as long as 3 hours, but no effect of standing in physiological salt solution for this time on unirradiated spores was observed either for the time required for germination or on the germinability.

The dosage of radiation given the spore suspension was calculated when the time of exposure, intensity of the beam of radiation, proportion of the beam absorbed by the suspension and the number of spores responsible for this absorption were known.

In order to determine the shape of the lethal curve, experiments were carried out in triplicate with *Rhizopus suinus*. Cultures which had been incubated for 4 days after inoculation of the nutrient were used as a source of spores for irradiation. Germination counts were made of spores in the hanging drops. The criterion of germination used required that the germ tube produced by a spore must equal the diameter of the spore in order that the spore be considered germinated. The dosage of radiation in ergs

absorbed by each spore was compared with the survival of spores in the suspension, and curves showing the relation of these two factors were plotted.

The curve of figure 1 shows the results of 3 experiments carried out under identical conditions. The smoothed curve is of sigmoid shape.

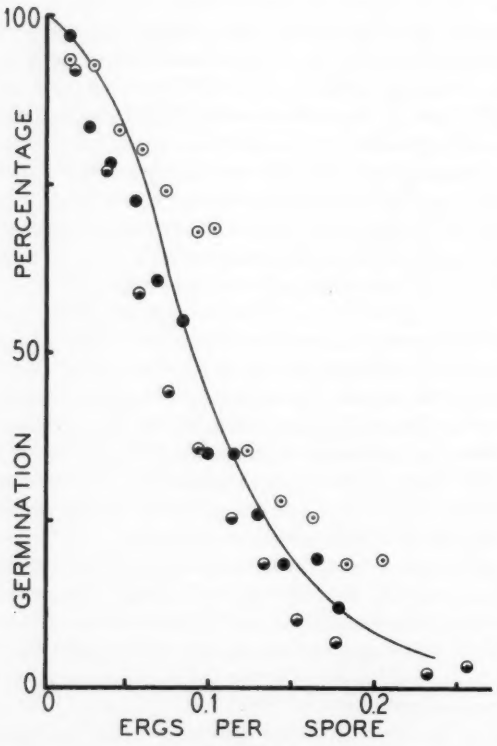

FIGURE 1

Composite curve of data of three experiments on the survival of spores from 4-day cultures of *Rhizopus suinus*.

A similar series of experiments was carried out using spores of the minus strain of *Mucor dispersus*. Spores for this series of experiments were similarly obtained from 4-day stock cultures. Results of 3 parallel experiments are presented by means of figure 2. As with *Rhizopus suinus*, the curve is sigmoid in shape.

Spores of *Aspergillus melleus* were likewise obtained from 4-day cultures. Suspensions to be irradiated were considerably more dense than the ones used for the mucoraceous fungi for the reason that the spores were much smaller and that absorption was less. Since these spores germinated in about the same time as those of the other species used, they were treated throughout in an identical manner. The results of a series of 3 experiments are presented graphically in figure 3. The survival curve for spores of *Aspergillus melleus* is of logarithmic form, and is quite different from that for spores of *Rhizopus* or *Mucor*. The significance of this behavior will be discussed later.

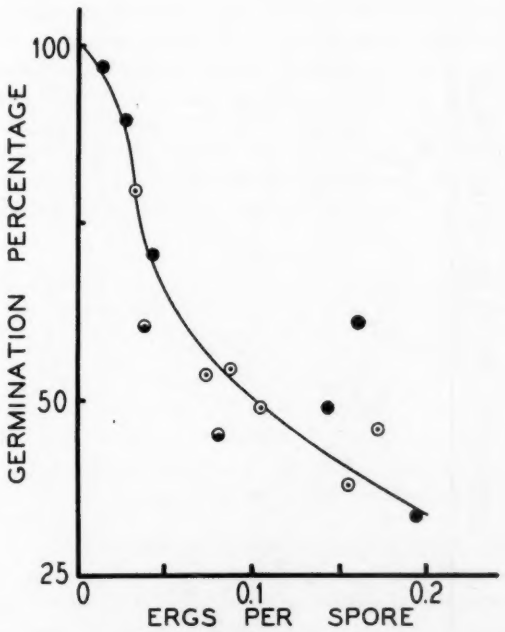

FIGURE 2

Composite curve of data of three experiments on the survival of spores from 4-day cultures of the minus strain of *Mucor dispersus*.

Another point of interest, shown by a comparison of figures 1, 2 and 3, is the difference in resistance of spores of the 3 species of fungi. Following a customary procedure, we shall compare the amounts of energy required for a 50% killing of the spores exposed to radiation, and for *Rhizopus suinus* this quantity is 0.09 erg per spore. The minus strain of *Mucor dis-*

persus, however, required a larger dosage of radiation, viz., 0.12 erg per spore, to cause the same amount of killing. *Aspergillus melleus*, on the other hand, is much the most susceptible of the three species employed, since 50% killing of the spores is effected by the absorption of 0.0064 erg per spore.

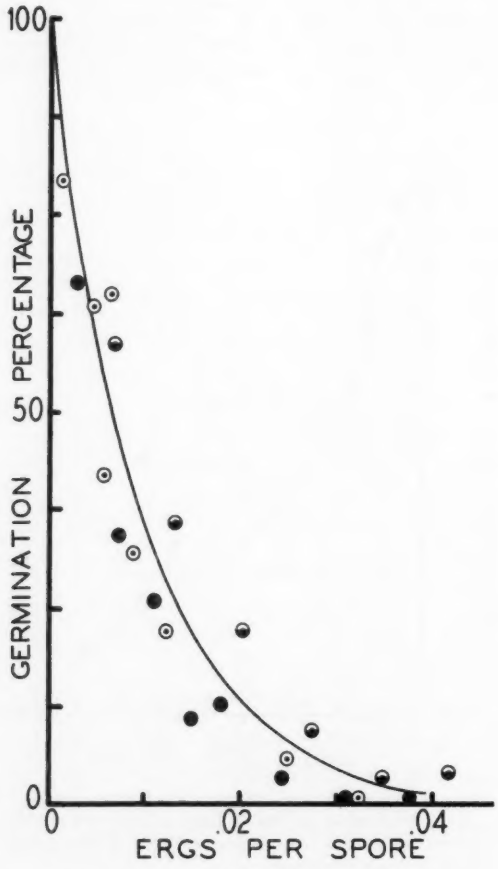

FIGURE 3

Composite curve of data of three experiments on the survival of spores from 4-day cultures of *Aspergillus melleus*.

The differences in resistance observed led the authors to make a quantitative comparison of the properties of the spores of these 3 species. Mea-

surements of the dimensions of large populations of spores grown in the standard manner were made by means of an ocular micrometer. Spores of *Rhizopus* were found to be of the size reported by Nielsen[10] in his original description of this fungus. They are of ellipsoidal shape and vary in size over a considerable range, from 5.5 to 9.0 μ by 3.5 to 7.0 μ. Those of *Aspergillus* were found to be of the size given by Thom and Church[9] and range from 2.5 to 4.0 μ in diameter, being spheroidal in shape. Spores of *Mucor* were by far the largest of the three, but showed a greater variation in size than is reported by Saccardo.[11] Spores were observed ranging from 9.0 to 15.4 μ in diameter and were spheroidal in form.

Since mucoraceous spores generally contain more than one nucleus, an attempt was made to determine the average number of nuclei in spores of *Mucor dispersus* produced under the conditions used for these experiments. Spores were stained by means of a modification of Feulgen's nuclear reaction and counts were made of the number of nuclei per spore in a population of spores. Counts of 9 to 20 nuclei per spore were obtained, and the average number of nuclei per spore was 13. Data comparing the properties of spores of the 3 species on the assumption that the spores of *Aspergillus* are uninucleate are given in table 1.

TABLE 1

A COMPARISON OF THREE SPECIES OF FUNGI WITH RESPECT TO RESISTANCE TO RADIATION, SIZE OF SPORES AND NUMBER OF NUCLEI PER SPORE

SPECIES	ERGS PER SPORE FOR 50% KILLING	(RATIO)	MEAN VOLUME OF SPORE (μ^3)	(RATIO)	NUCLEI PER SPORE
Aspergillus melleus	0.0064	1.0	8	1.0	1 (?)
Rhizopus suinus	0.088	13.7	28	3.4	
Mucor dispersus	0.12	17.5	113	14.1	13

The ratios recorded in the table were obtained by setting the values for spores of *Aspergillus melleus* equal to 1 and by raising the corresponding values for spores of the other fungi in proportion.

From this table it is seen that the differences in resistance between species is not correlated directly with the volume of the spore or with the number of nuclei. Very possibly differing degrees of resistance between spores of various species are correlated not only with the size of spores and the number of nuclei contained, but also with the kind and amount of pigmentation in the spore wall.

Differences in the types of killing curves obtained for *Aspergillus* and for the mucoraceous species used in this study merit some consideration. The killing curves recorded in the literature have been variously interpreted as indicative of a multiple quantum hit-to-kill relation and as indicative of variation in the susceptibility to radiation on the part of a population of spores. Other workers have postulated an ideal shape of killing

curve as characterizing a unimolecular reaction. This would involve a logarithmic order of death such as was obtained for *Aspergillus melleus* (Fig. 3). Departures from this type of curve have been explained as being attributable to various factors, such as the amount of pigmentation of spores, the temperature at which irradiation was carried out, age of the spores and so on. Factors such as the size of the spores and the number of nuclei which they contain may also influence the shape of the survival curve.

If it is assumed that the nucleus is the part of the cell which must be hit by quanta in order that death may result, any factors which reduce the number of quanta hitting the nucleus will also influence the effectiveness of an injurious wave-length. The volume of protoplasm about the nucleus might act in this way by absorption of quanta, and through its action on the nucleus might alter the shape of the survival curve.

In the case of multinucleate spores the question arises whether or not more than one nucleus need survive irradiation in order that the spore may germinate. Since a population of mucoraceous spores shows a wide variability with respect to the number of nuclei per spore, there seems no *a priori* reason for believing that more than one nucleus need survive irradiation in order to insure normal germination of the spore, even though growth in the early stages may be somewhat retared as a result of the treatment. If this is the case, it may well be that ability of the spore to survive treatment, although all but one of the contained nuclei are inactivated, may alter the shape of the survival curve. When the spore contains a large number of nuclei, the centrally located nuclei are more protected, and the probability is much less that a quantum entering the spore will hit one of them than is the probability that the quantum will hit one of the outer nuclei. The effect of inactivation of one or more of the nuclei in a spore on the subsequent behavior of the spore is unknown.

The work of Smith,[2] referred to in the introduction, may provide an explanation for the difference in the shape of the survival curves obtained for *Aspergillus* in the present study as contrasted with the shape of survival curves obtained in other studies on uninucleate fungous spores. She, working with *Fusarium*, and Hollaender and Emmons,[4] with *Trichophyton*, obtained sigmoid survival curves at irradiation temperatures below 20°C. If spores of both these fungi are uninucleate, results obtained with spores of *Aspergillus* in the present study might be expected to be similar to those obtained by these authors. At irradiation temperatures lying between 20° and 50°C., Smith obtained a gradual transition from sigmoid to logarithmic shape of the survival curve. In the present investigation, irradiation was carried out at a temperature of 25°C. This was somewhat higher than the temperature at which irradiation was carried out by Smith and by Hollaender and Emmons, and the possibility exists

that the higher temperature at which irradiation was carried out was responsible for the logarithmic shape of the survival curves for spores of *Aspergillus*. Zahl, Koller and Haskins[12] have recently given results of irradiation of spores of *Aspergillus niger* showing survival curves of a transition type between sigmoid and logarithmic shape. The temperatures employed are not stated.

The relation between resistance to radiation and the age of spores was studied, using cultures of *Rhizopus suinus*. The data of a previous experiment were used to represent the behavior of a 4-day culture. Spores from cultures which had been incubated 12, 14, 21 and 34 days after inoculation were irradiated and data on percentage germination resulting from this treatment were accumulated in the manner described. The germination obtained in control hanging drops of older cultures was less than in the younger cultures, owing to loss of viability of spores. Consequently, the germination percentages of control hanging drops were in all cases regarded as 100, and the values for irradiated suspensions of spores from these cultures were raised in proportion.

The results of these experiments are presented in table 2. The table shows how resistance to radiation increases with age of the culture from which the exposed spores are taken. These results confirm those of Oster,[3] and are in disagreement with the results of Schreiber,[7] both of whom used yeast as the test organism. The resistance does not change markedly during the first few days of growth; the difference in the table for cultures 4, 12 and 14 days old probably are not in themselves significant. The trend is definite, however, and extends throughout the series.

TABLE 2

THE RELATION BETWEEN RESISTANCE TO RADIATION AND AGE OF SPORES OF *Rhizopus suinus*

AGE OF CULTURE (DAYS)	ERG PER SPORE FOR 50% KILLING
4	0.075
11	0.08
14	0.09
21	0.11
34	0.21

Increased resistance to radiation with age may be caused by a number of factors. The spores may continue to increase somewhat in size after being formed in the sporangium. Any considerable increase in size not accompanied by nuclear division would result in increased resistance of the spore to radiation. The mass color of spores of *Rhizopus suinus* deepens with age, and an increased amount of pigment would also increase resistance to radiation. Possibly the drying of spores with age alters the protoplasm in such manner that greater protoplasmic absorption of radiation occurs with resultant decrease in nuclear absorption.

Summary.—Spores of the fungi *Rhizopus suinus*, *Mucor dispersus* and *Aspergillus melleus* were tested with respect to their resistance to ultraviolet of wave-length 2650 A. Sigmoid survival curves were obtained for the fungi *Rhizopus suinus* and the minus strain of *Mucor dispersus* when spores from 4-day cultures were irradiated. Survival curves describing a logarithmic order of death were exhibited by spores from 4-day cultures of *Aspergillus melleus*.

The three species of fungi showed marked differences in susceptibility to radiation (2650 A). A combination of the differences in pigmentation, size of the spores and the number of nuclei may account for the differences in susceptibility observed.

A trend of increasing resistance to radiation with increased age was observed for spores of *Rhizopus suinus*.

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PROLYCOPENE, A NATURALLY OCCURRING STEREOISOMER OF LYCOPENE

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In this paper we record the observation that there occurs in the variety of tomato called "tangerine tomato" a carotenoid, *prolycopene*, which is an isomer of lycopene; the isomeric relationship is similar to that between lycopene and neolycopene,[1] and in our opinion prolycopene is to be classed as a naturally occurring neolycopene, being the first observed natural *neo* form of a C_{40}-carotenoid.

The color of ripe tomato fruits is determined primarily by two sets

of genes, one of which, *Y* and *y*, affects the color of the skin, and the other, *R* and *r*, the color of the flesh.[2] The relation between these genes and the carotenoid pigments of the fruit has been studied.[3] In addition to the red genes *R* and *r* there is only one other pair of genes known which affects the flesh color of ripe tomato fruits.[4] This pair has been named "tangerine," *T* and *t*. In double recessive form *tt* it causes a brilliant orange color in the fruits which would have been red if the dominant gene *T* had been present. The two genes *R* and *T* are inherited independently and are located in chromosomes 2 and 7, respectively.

The tangerine tomatoes (a commercial variety) used in the present investigation were grown in a greenhouse kept at 26.5°C. during the day and 19°C. during the night. The conditions of temperature and humidity were the same as for red tomatoes studied earlier,[5] and the corresponding data of this paper and the earlier paper are directly comparable.

The tangerine tomato was mashed in a mortar and shaken with successive portions of methanol and petroleum ether (b. p. 60–70°) which were in such ratios that two liquid phases were present. The orange-yellow extract was washed free of methanol with water, dried over sodium sulfate, concentrated in vacuum and chromatographed on calcium hydroxide (Shell). The time required for the entire process up to the chromatographing was about two hours. By development with petroleum ether and benzene (1:1) or, better, with petroleum ether containing 10% acetone a chromatogram is obtained containing over a dozen layers (table 1). Lycopene is found near the top (layer 1), and neolycopene underneath it (layer 2). After the next minor pigment layers the main layer (layer 9), that of the new pigment prolycopene, appears, followed by several smaller layers and finally by β-carotene and its isomers (layers 13 to 15).

The main layer was eluted with petroleum ether and ethanol, washed free of ethanol, and dried. The solution was re-chromatographed after it had stood at 5° for one day. The new chromatogram showed about nine layers in addition to the main one. (Lycopene itself on similar isomerization gives a chromatogram with three preponderant layers.) These layers were similar to some of those (1 to 8, 10) in the original chromatogram, including layer 1, that of lycopene, which suggested that the new pigment had, on standing, in part isomerized to lycopene. A portion of a petroleum-ether solution of the main layer was then treated in the cell of a spectroscope (Evaluating Grating Spectroscope, Loewe-Schumm design, Carl Zeiss, Jena; 2-mm. Jena light filter No. BG-7) with an iodine solution (in petroleum ether), and the spectrum was observed to change very rapidly (within a second or two) from that of the original pigment (469, 441 mμ) to a more intense spectrum apparently identical with that of lycopene after similar treatment (502, 471, 441 mμ). The assumption that lycopene is the

preponderant component of the resulting pigment mixture was confirmed by adsorption analysis; the main layer of the chromatogram was lycopene,

TABLE 1

SPECTRA IN PETROLEUM-ETHER SOLUTION OF INDIVIDUAL PIGMENTS OF THE TANGERINE TOMATO, AND SPECTRA AFTER REACTION UNDER THE CATALYTIC INFLUENCE OF IODINE. THE PIGMENTS ARE IN THE SEQUENCE OBSERVED IN THE CALCIUM HYDROXIDE CHROMATOGRAM

LAYER NUMBER	SPECTRUM BEFORE CATALYSIS				SPECTRUM AFTER CATALYSIS			
1	505	475	446	(420)	502	471	441	(413) mμ
2	499.5	468	349		502	471	441.5	
3	498.5	468			501.5	471.5	441.5	
4	498.5	474.5	447		502	470.5	442	
5		475.5	445.5		502	471.5	441	
6		472	443.5	(417)	502	471	441.5	
7	497.5	469	438.5		502	471.5	440.5	
8	495.5	468	437.5		499	470.5	439.5	
9		468.5	441		502	470.5	441	
10		464.5	431		501	470.5	435.5	
11	(490.5)	(462.5)	430		(497)	470.5		
12		(Traces)				(Traces)		
13	486	455.5	427		485.5	456.5		
14	487	455			484	453.5		
15	481	450.5			483.5	452.5		

as verified by its spectrum (505, 475, 446 mμ) and by the mixed chromatogram method (a mixture of a solution of this layer and of a sample of pure lycopene was not separated in the chromatographic column). The other products of the catalytic isomerization are to be classed as neolycopenes.

The same spectral change was observed also to occur, more slowly than with iodine, under the catalytic influence of sulfur or hydrogen bromide in petroleum ether.

In another experiment the total extract (partially isomerized by standing) of the tangerine tomato was chromatographed, the individual layers were cut out and eluted, and their spectra in petroleum ether were determined. Then iodine was added to each solution and after a few minutes (at 30°) the spectra were again determined. The wave-lengths obtained in this experiment are given in table 1. It is seen to be probable that all of the pigments 2 to 11 are stereoisomers of lycopene; layer 2 is certainly the previously described neolycopene.[1]

Pending the establishment in detail of the stereochemical relations among the numerous isomers of lycopene, we have assigned the name prolycopene to the pigment described above, which is the main pigment of ripe tangerine tomato fruits. The results of our investigation suggest that the gene *R* may be involved in the synthesis of prolycopene in both tanger-

ine tomatoes and red tomatoes, and the gene *T* may then be responsible for the conversion of prolycopene into lycopene in red tomatoes.

The fact that the same catalysts are effective suggests that the relation between lycopene and prolycopene is similar to that between the carotenoids and their labile isomers, which have been under investigation recently.[6, 7, 1, 8, 9] Prolycopene has especial interest as the first naturally occurring C_{40}-carotenoid which gives rise to isomers which absorb light more intensely than itself and at longer wave-lengths. It is also interesting that the principal product of its isomerization is a well-known naturally occurring pigment.

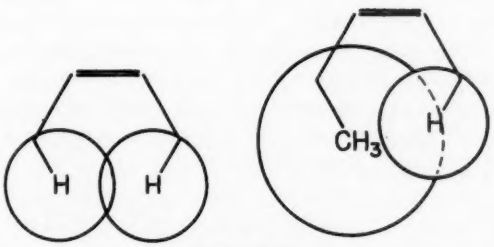


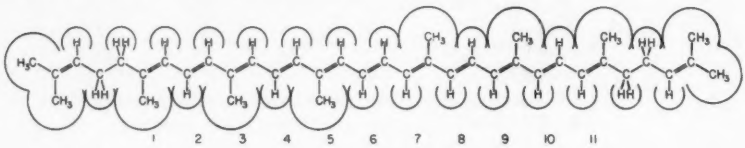
FIGURE 1

Drawings showing overlapping of hydrogen atoms in —CH—CR=CR—CH— and of hydrogen and methyl in —CH—CR=CR—CCH_3— with *cis* configuration.

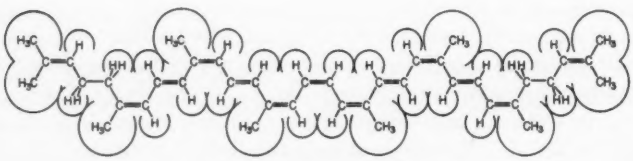
It now seems very probable that the suggestion[1] that the isomerization of the carotenoids is geometrical isomerization about the conjugated double bonds is essentially correct; the alternative suggestion[7] of double-bond migration must be abandoned as a general explanation because of the impossibility of accounting for the large number of the observed lycopene isomers on this basis. Experiments which are being carried on in these laboratories by Dr. A. Polgár have already shown that β-carotene also forms a larger number of isomers than can be explained by double-bond migration. We have, moreover, found that lutein, containing an α-ionone ring, is not converted into zeaxanthin, containing a corresponding β-ring, by treatment with iodine, which suggests that iodine is not effective as a catalyst for such double-bond migration.

Aside from the fact that the effective catalysts are known to catalyze *cis-trans* isomerizations, an argument based on the intensity of light absorption has been presented.[10] Another argument, as follows, may be based on the spectral shift, which is observed to be toward shorter wave-lengths for the *neo* forms. The theory of resonance requires that for effective conjugation the configuration about the single bonds as well as the double bonds of the system be coplanar.[11] The hydrogen atoms of CH

groups adjacent to a double bond with a coplanar *cis* configuration overlap somewhat, as shown in figure 1, where they are drawn with the normal van der Waals radius 1.0 A. (A smaller radius, 0.85 A, was used in drawing figure 10 of reference 10.) This overlapping and consequent interatomic repulsion would have two significant effects. First, it would decrease the stability of *neo* isomers (containing *cis* configurations) relative to the completely *trans* normal form, explaining the observation that in an equilibrium mixture the amount of the carotenoid possessing the spectrum with largest wave-length exceeds that of any one *neo* isomer.[12] Second, the repulsion would tend to push the *cis* molecule out of complete coplanarity, and thus to interfere to some extent with the conjugation and to shift the absorption maxima to shorter wave-lengths. Many *neo* isomers have maxima shifted by 5 to 7 mμ from those of the normal carotenoids; this we interpret as due to the interference by one *cis* bond with the conjugation. Zeaxanthin and capsanthin have also *neo* forms shifted by 10 or 12 mμ, and taraxanthin one shifted by 21 mμ, which we may interpret as resulting from two and three (or four) *cis* bonds, respectively.



LYCOPENE



PROLYCOPENE

FIGURE 2

Suggested stereochemical structures of lycopene and prolycopene.

The wave-length difference of 36 mμ between lycopene and prolycopene we would attribute to the presence in prolycopene of the *cis* configuration for as many as five or seven of the eleven conjugated double bonds. As shown in figure 1, steric interaction of hydrogen and methyl prevents the assumption of the *cis* configuration by double bonds with C—CH_3 adjacent;[10] i.e., those participating in the grouping C=C—C , these being
CH_3

the second, fourth, eighth and tenth double bonds of the series of eleven in the conjugated system of lycopene (Fig. 2). The suggestion advanced with some reserve by Karrer and Solmssen[13] that the double bond which differentiates labile and stable bixin is the third from the free carboxyl group (corresponding to number 8 of lycopene, figure 2) is incompatible with our arguments; this bond is of the type for which the *cis* configuration is forbidden.

It may be pointed out that not more than one double bond in each isoprene unit can assume the *cis* configuration, this being the bond adjacent to the methyl side chain. In addition, the central double bond (the one of β-carotene, for example, which is split during its conversion in the body into vitamin A) can also assume this configuration. The stereochemical structure suggested in figure 2 for prolycopene predicates that all seven double bonds for which this is possible are in the *cis* configuration, making prolycopene one extreme in the series of which the completely *trans* lycopene is the other. On isomerization prolycopene would not be converted directly into lycopene, but would pass through intermediate isomeric forms, *cis* about fewer than seven double bonds, as rotation occurred about the bonds one by one. The total number of possible *cis-trans* isomers for the seven stereochemically effective double bonds in the chain with equivalent ends is 72; in our small-scale chromatograms about a dozen zones have been observed, some of which may contain two or more components. Further support for the idea that prolycopene passes through intermediate *neo* forms during conversion into lycopene is given by the observation that the ratios of amounts of *neo* forms to lycopene in partially isomerized prolycopene are greater than for the mixture of isomers obtained by the isomerization of lycopene.

TABLE 2

NUMBERS OF *cis-trans* ISOMERS FOR UNSYMMETRICAL AND SYMMETRICAL CHAINS CONTAINING *n* STEREOCHEMICALLY EFFECTIVE DOUBLE BONDS

UNSYMMETRICAL CHAINS		SYMMETRICAL CHAINS	
$n = 1$	2 isomers	$n = 1$	2 isomers
2	4	2	3
3	8	3	6
4	16	4	10
5	32	5	20
6	64	6	36
7	128	7	72
8	256	8	136
9	512	9	272
10	1024	10	528
11	2048	11	1056
12	4096	12	2080

It is of interest in connection with our program of identifying the geometrical isomers of the carotenoids to consider the numbers of possible isomers. The chains are for this purpose divided into two classes: those, XX', whose two halves are not equivalent (called unsymmetrical chains) and those, XX, whose halves are topologically equivalent (called symmetrical chains). The formulas for the number of isomers for n effective double bonds (able to assume *cis* and *trans* configurations which are not equivalent) are the following: unsymmetrical chains, $N = 2^n$ for n odd or even; symmetrical chains, $N = 2^{(n-1)/2}(2^{(n-1)/2} + 1)$ for n odd, $N = 2^{(n/2)-1}(2^{n/2} + 1)$ for n even. Numerical values are given in table 2. It is seen that 10 isomers are expected for azafrin (unsymmetrical, $n = 4$); 32 for α-carotene, kryptoxanthin, lutein, capsanthin, semi-β-carotenone, citraurin and bixin (unsymmetrical, $n = 5$); 20 for β-carotene, zeaxanthin, astacin, capsorubin, rhodoxanthin, β-carotenone, norbixin, methylbixin and crocetin (symmetrical, $n = 5$); 64 for γ-carotene and rubixanthin (unsymmetrical, $n = 6$); 128 for lycoxanthin and rhodoviolascin (unsymmetrical, $n = 7$); and 72 for lycopene, as mentioned above (symmetrical, $n = 7$).

* Contribution No. 851.

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RADIOACTIVE CARBON AS AN INDICATOR OF CARBON DIOXIDE UTILIZATION. VI. ON THE POSSIBILITY OF CARBON DIOXIDE REDUCTION VIA THE CARBOXYLASE SYSTEM

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Recent experiments,[1] carried out with the aid of labeled carbon, have shown beyond any doubt that CO_2 enters into the metabolism of a large variety of organisms in the absence of light. As a consequence it has been suggested that CO_2 assimilation may be a process of wide-spread occurrence in living systems.

The metabolic processes of living organisms are for the most part enzyme catalyzed. The recent studies of Virtanen, Woods, Borsook, Warburg, Cori and co-workers, Hanes and many others[2] have clearly demonstrated that many of these processes are reversible, and there is at present no reason for assuming that this is not true in all cases. It is generally believed that the enzymatic decomposition of keto acids accounts for the greater part of the CO_2 produced by living organisms. If these latter reactions are reversible, they could be a mode of entry of CO_2 into cellular biochemical processes.[3] With this point in mind we have investigated the decarboxylation of pyruvic acid using radioactive carbon (C^{11}) as a tracer.[4]

The enzyme carboxylase is known to catalyze the decomposition of pyruvic acid with the production of CO_2 as one of the end-products. This enzyme is of wide-spread occurrence among living organisms. Furthermore, cell-free preparations which will function *in vitro* can be easily prepared and its action on pyruvic acid has been carefully studied. It is known that the decarboxylation of pyruvic acid gives rise to CO_2 and acetaldehyde

$$CH_3CO\cdot COOH \longrightarrow CH_3CHO + CO_2 \qquad (1)$$

There has been as yet no experimental evidence to show whether this reaction is reversible However, assuming the reaction to be reversible, the free energy change (ΔF°) can be estimated from the available thermodynamic data[5] to be approximately $-10{,}000$ calories for the pure substances in aqueous solution. Therefore it is apparent that the reverse action (if it occurs) will be exceedingly slow compared to the decarboxylation reaction, so that a very sensitive indicator must be employed in order to detect it.

The reformation of pyruvic acid has been studied using C^*O_2. In an experiment to be described in detail below, inactive pyruvic acid was decarboxylated enzymatically in the presence of inactive CH_3CHO and C^*O_2 of very high specific radioactivity. However, no conclusive evidence (presence of radioactive keto-acids) for the reversibility of the decarboxylation could be obtained because the rate of decarboxylation in the presence of carboxylase was too fast. It was therefore necessary to enhance the possibility of pyruvate-synthesis by slowing down the decarboxylation.

It has been found that the rate of decarboxylation can be decreased by the addition of acetaldehyde.[9] The effects of various concentrations of acetaldehyde on the rate of this reaction were tested in experiments conducted with the usual Warburg technique. It was found that the pyruvate decarboxylation was reversibly inhibited by CH_3CHO in concentrations up to $\sim$0.4 *M*. Concentrations of CH_3CHO greater than this destroyed the enzyme by denaturing the soluble protein and precipitating it as a milky white solid. Enzyme solutions which had been partially inactivated by concentrations of acetaldehyde up to 0.4 *M* could be restored to very nearly complete activity by removing the acetaldehyde through aeration, thus demonstrating the inhibition to be reversible. Coryell, Heegaard, *et al.*,[6] advanced an explanation for the inhibiting effect of acetaldehyde on the decarboxylation of pyruvic acid by assuming a competition of the two substances for the same active group of the enzyme. They suggest also that the inhibition may be enhanced in the early stages by adding acetaldehyde some 20 minutes before the pyruvate is added to fill most of these active groups with acetaldehyde, since their experiments indicate that the replacements at the active positions are slow. Therefore the acetaldehyde together with the C^*O_2 was added $\sim$20 minutes before the pyruvate in these experiments.

The enzyme solution was prepared in the following way: Bottom yeast was dried at 30°C., finely powdered and allowed to autolyze in the presence of three parts of distilled water for three hours at 35°C.[7] The autolysate was then centrifuged and the residue discarded; the clear supernatant solution decomposed pyruvate with great rapidity when used in phosphate buffer at pH 6.0 to 6.2.

The radiocarbon experiments were carried out in the following manner: Into each of two vessels were introduced 2.0 ml. of enzyme solution in *M*/5 phosphate buffer at pH 6.0; 1.5 ml. of a 0.9 *M* solution of acetaldehyde were added to each vessel, which brought the final concentration of aldehyde to 0.39 *M*. The C^*O_2 was introduced, and the vessels were shaken at 30°C. for 20 minutes, after which time 0.3 ml. of a sodium pyruvate solution (60 mg. sodium pyruvate) was added to each vessel. The concentration of pyruvate was thus 0.14 *M*. One vessel was shaken at 30°C. for 10 minutes, the other for 50 minutes.

For the measurement of the amount of radioactive keto acid formed, the solution from each vessel was divided into halves which were treated as follows:

(1) To one portion, 0.2 g. of sodium pyruvate and 0.1 g. of $NaHCO_3$ were added as carriers, the solution was acidified to pH 1.0 with HCl, and boiled gently to expel C^*O_2, after which HCl and $NaHCO_3$ were again added and the boiling was repeated. The moderate amount of protein which precipitated was removed by centrifugation; no radioactivity could be detected in this precipitate. The pyruvic acid in the clear supernatant solution was precipitated as the 2,4-dinitrophenylhydrazone, which was filtered, washed and dissolved in Na_2CO_3. The hydrazone was reprecipitated with HCl, and was found to be radioactive (table 1).

Because it seemed of interest to determine whether the keto acid formed in this reaction was present in combination with the enzyme, a different treatment was used with the second portion. It was conceivable that the boiling with acid may have dissociated the enzyme-substrate complex. For this reaction both high temperature and acidification were avoided in eliminating the C^*O_2, which was achieved in the following manner.

(2) To the second portion, 0.2 g. of sodium pyruvate and 0.2 g. of Na_2CO_3 were added as carriers, followed by excess $CaCl_2$ and NaOH. The precipitate of CaC^*O_3 was removed by filtration and this procedure was repeated with the filtrate. Control experiments had shown that this method was effective in completely removing C^*O_2. The clear filtrate was acidified and the 2,4-dinitrophenylhydrazone of pyruvic acid was precipitated. This hydrazone was only slightly active (table 1).

TABLE 1

TIME OF DECARBOXYLATION (MINUTES)	RADIOACTIVITY[a] FOUND IN HYDRAZONE FROM:	
	Na_2CO_3 treatment	HCl extraction
10	5 ± 4 counts/min.	86 ± 4 counts/min.
50	14 ± 10 counts/min.	480 ± 20 counts/min.

[a] The C^*O_2 introduced into each vessel had an activity of 1.4×10^6 counts/min. All figures are corrected for the decay of C^{11} to the time of counting the first hydrazone sample.

A control experiment was performed by shaking a solution of $CH_3COCOOH$ and CH_3CHO with C^*O_2 for 30 minutes. The carbon dioxide was removed by gentle boiling with dilute HCl. The CH_3CHO and $CH_3COCOOH$ were brought down with 2,4-dinitrophenylhydrazine and carefully washed. This hydrazone precipitate was inactive (< 3 counts/minute[a]).

Table 1 shows that the quantity of C* in the pyruvate precipitate is very small. Indeed, when the rate of decarboxylation was not strongly inhibited by the addition of sufficient CH_3CHO, the radioactivity was too small to detect.

It may be concluded from table 1 that a very small but nevertheless definite amount of a labeled carbonyl compound (probably pyruvic acid) has been formed from C^*O_2. The significant difference in the radioactivities of the pyruvate precipitates obtained in procedures (1) and (2) is interesting. It would appear that most of the labeled pyruvate is bound to the catalyst and is split off by boiling with HCl (method 1). The enzyme was completely precipitated with the $CaCO_3$ (method 2), carrying down with it the major part of the labeled pyruvate.

The amount of C^*O_2 used by this reaction is far too small to account for the CO_2 utilization observed in the many systems thus far studied. It may be, however, that *in vivo* the labeled pyruvate could be transformed or removed from the enzyme surface by rapid reactions competing with the decarboxylation.

We have investigated the possibility of rapid competing reactions *in vivo* using the homofermentative lactic acid bacterium *Streptococcus lactis*. It is known that this organism carries out a dismutation of pyruvic acid which results in the formation of CO_2, acetic and lactic acids according to the equation:[8]

$$2CH_3CO\cdot COOH + H_2O \longrightarrow CO_2 + CH_3COOH + CH_3CHOH\cdot COOH \qquad (2)$$

This net equation may be considered as consisting of at least two separate reactions:

$$CH_3CO\cdot COOH + H_2O \longrightarrow CH_3COOH + CO_2 + 2H^+ + 2e^- \qquad (3)$$

and

$$CH_3CO\cdot COOH + 2H^+ + 2e^- \longrightarrow CH_3CHOH\cdot COOH \qquad (4)$$

If these reactions are allowed to proceed in the presence of C^*O_2, the labeled pyruvate formed by the reversal of (3) could be reduced to lactate and thus stabilized. In that case the lactic acid should contain C*.

Fermentations were carried out anaerobically in the presence of C^*O_2 using sodium pyruvate and also dextrose as substrates in separate vessels. *Strep. lactis* ferments dextrose according to the equation:

$$C_6H_{12}O_6 \longrightarrow 2CH_3CHOH\cdot COOH \qquad (5)$$

The organisms were grown in yeast autolysate diluted 1 to 5 in $M/5$ phosphate buffer at pH 7.0. The cultures were incubated at 28°C. for 24 hours and showed a high rate of metabolic activity, as evidenced by the rate of acid production from dextrose in Warburg experiments.

Heavy suspensions of the bacteria were prepared for the radioactive carbon experiments by centrifuging the cells out of a large amount of

growth medium, and washing them twice in tap water. The organisms were suspended in $M/5$ phosphate buffer at pH 7.0 and placed in the experimental vessels. Dextrose was added to one vessel and sodium pyruvate to another in such amounts that the final concentration of the substrate was 2%. C^*O_2 was admitted and the suspensions were shaken under anaerobic conditions at 30°C.

At the close of the experiment, inactive pyruvic, lactic and acetic acids were added as carriers. C^*O_2 was quantitatively expelled by the addition of inactive $NaHCO_3$ and acid followed by gentle boiling, and the cells were then removed by centrifugation. Pyruvic acid was isolated from the supernatant liquid as the 2,4-dinitrophenylhydrazone, which was removed by filtration. The acetic acid was separated from the lactic acid by steam distillation; the non-volatile residue was subjected to a frational vacuum distillation to remove lactic acid. The distillation was followed both by titrimetric and radioactivity measurements. The results are summarized in table 2.

TABLE 2

C^*O_2 UTILIZATION BY *Streptococcus lactis*

SUBSTRATE	DURATION OF EXPERIMENT (MIN.)	C* (COUNTS/MIN.)[a] FOUND IN: SUPERNATANT EXTRACT	CELLS	PYRUVIC ACID	LACTIC ACID	ACETIC ACID
$CH_3COCOONa$	60	4.5×10^4	0.1×10^4	$<0.04 \times 10$	$<0.04 \times 10^4$	$<0.01 \times 10^4$
$C_6H_{12}O_6$	30	0.44×10^4	0.4×10^4	.....	$<0.006 \times 10^4$	.....

[a] The C^*O_2 introduced into each vessel had an activity of 5×10^6 counts/min. All values are corrected for decay of C^{11} to the time of counting the first sample.

It is apparent from the above table that the bacteria have assimilated small amounts of C^*O_2. Nevertheless, the formation of labeled pyruvic, lactic and acetic acids could not be detected. Due to the low radioactivity of the extracts and the short half-life of C^{11}, the identity of the radioactive substances formed was not determined.

In conclusion, then, the *in vitro* experiments conducted with carboxylase preparations have shown that in the presence of acetaldehyde and pyruvate a very small amount of C^*O_2 is utilized, resulting in the formation of a radioactive carbonyl compound. In addition, *in vivo* experiments with *Strep. lactis* would seem to indicate that the reformation of pyruvate from CO_2 is not responsible for the entrance of CO_2 into the biochemical processes of this organism.

It is a pleasure to express our thanks to Professors C. B. Van Niel and H. A. Barker for valuable advice and discussion, and to Professor E. O. Lawrence and members of the Radiation Laboratory for their coöperation.

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*VISIBILITY OF THE PRIMARY SPINDLE FIBRES AND THE COURSE OF MITOSIS IN THE LIVING BLASTOMERES OF THE MITE, PEDICULOPSIS GRAMINUM (REUT.)**

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The frequent observation that mitotic spindles of living, untreated cells are optically homogeneous, as well as Chambers'[1] inability to demonstrate fibres with his microdissection methods, have led to a widespread opinion among biologists that spindle fibres have no morphological reality as such. They are commonly held to be no more than coagulation artifacts produced by fixation, in contrast to the easily visible astral rays of living material such as the *Arbacia* egg.

Opposed to this interpretation are the results of experiments by Bělař[2] and Schrader,[3] and Schmidt's[4] recent demonstrations of optical anisotropy

of the spindles of living sea urchin eggs. Indeed these observations strongly suggest that truly fibrous structures comprise the spindle elements in dividing cells, but visual demonstration of such fibres in untreated living material appears to be still wanting.[5]

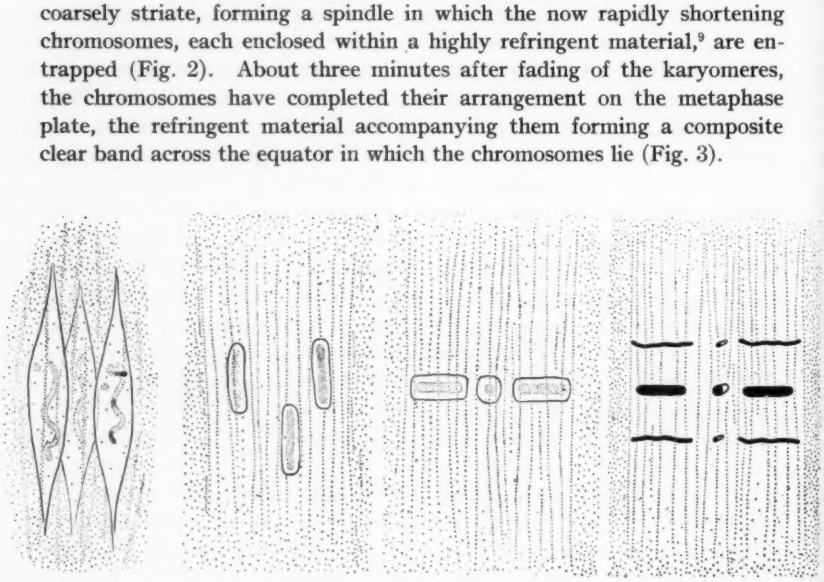
The present observations provide the crucial datum regarding the morphological reality of spindle fibres, for continuous or primary spindle fibres may be seen with ordinary light in the dividing blastomeres of the living, untreated eggs of *Pediculopsis graminum*. Astral rays cannot complicate the picture, for both centrioles and asters are lacking. Thread-like mitochondria, which so often impart a spurious fibrous appearance to the spindle of the living insect spermatocyte,[6] are likewise absent. Janus Green B, Benda, and Bensley mitochondrial techniques give results agreeing fairly well with Das's[7] recent work on the egg of the tick or mite, *Rhipicephalus*. The mitochondria of the *Pediculopsis* egg appear as minute spheroids and stubby rods which grade into small and large "yolk" granules taking mitochondrial stains. The mitochondria do not appear to assume any special arrangement with respect to the spindle, and clearly are not the cause of the fibrillation or streaks seen in the living spindle of the early blastomeres of *Pediculopsis*.

Eggs of the mite *Pediculopsis* in fifth to eight cleavage are mounted in hanging drops of body fluid from the mother, covered in turn by a drop of paraffin oil. The whole preparation is then studied by transmitted light, the optics employed being a Zeiss pancratic condenser, Zeiss 3 mm. N.A. 1.4 objective, in conjunction with Zeiss K 15× and K 20× oculars.

As has been shown in an earlier work,[8] in fixed and stained preparations the six chromosomes of the diploid eggs each lie in a separate vesicle or karyomere. These vesicles elongate in prophase, the chromosomes condensing within them. At prometaphase the outlines of the greatly elongated karyomeres are lost, leaving the chromosomes imprisoned within the sheafs of primary spindle fibres which form about the region of the karyomeres. The chromosomes become aligned on the metaphase plate; the sister chromatids separate and elaborate between them Feulgen-negative replicas of the former single chromosomes. Thereafter the chromosomes undergo parallel displacement to the poles of the spindle, leaving the stationary chromosome replicas or *chromosomoids* at the equatorial plate (Fig. 4). At the spindle poles each chromosome vesiculates, swells and is transformed into an individual karyomere. During this process a nucleolus appears in each karyomere. The cleavage plane passes through the equator and is foreshadowed by the dwindling away of the enigmatic chromosomoids.

In the living blastomere elongation of the karyomeres may easily be followed, and the chromosome and nucleolus within each discerned with some difficulty (Fig. 1). Shortly after disappearance of the nucleoli of the

greatly elongated karyomeres, the karyomeres themselves fade from view. At this time the surrounding, yolk-free cytoplasm becomes more and more coarsely striate, forming a spindle in which the now rapidly shortening chromosomes, each enclosed within a highly refringent material,[9] are entrapped (Fig. 2). About three minutes after fading of the karyomeres, the chromosomes have completed their arrangement on the metaphase plate, the refringent material accompanying them forming a composite clear band across the equator in which the chromosomes lie (Fig. 3).



1 2 3 4

FIGURES 1–4

Figure 1.—Late prophase; cytoplasm beginning to show centroplasmic striae, chromosomes and nucleoli visible within the elongated karyomeres. Figure 2.—Prometaphase; chromosomes (stippled) enclosed within refringent "capsules," spindle fibres visible. Figure 3.—Metaphase; chromosomes still within refringent capsules, spindle fibres visible. Figure 4.—Anaphase, showing parallel displacement of sister chromosomes and equatorially situated chromosomoids.

All figures are conventionalized and include only the yolk-free central areas of the blastomeres and haploid nuclear components. Figures 1, 2 and 3 are from freehand sketches of mitoses in living blastomeres. Figure 4 is from a fixed and stained preparation.

Here they remain with no observed change for about three and one-half minutes, after which a confusing array of changes takes place. The chromosomes become clearly double, but no chromosomoids have been observed between sister chromatids. The sharp outlines of the refringent materials in which the chromosomes appear embedded are lost. The continuous spindle fibres appear to fade momentarily, but suddenly come back to view more distinct and coarse than before. At this time the equator may show a number of discrete, refringent bodies (chromosomoids ?), but the chromosomes have been lost to view in the spindle. About three

minutes following "doubling" of the chromosomes, the cleavage plane cuts rapidly through the equator and small yolk granules drift into this area obscuring most of the central details. Nevertheless the spindle fibres are still visible; shortly thereafter they fade from view. The telophase karyomeres are to be seen at the poles generally within two to three minutes following cleavage. They grow rapidly and apparently undergo no passive interphase, for from the first they continue to elongate in preparation for the succeeding cleavage. The nucleolus is visible within eight minutes of cleavage—often earlier. The fading of the nucleolus anticipates the disappearance of the karyomeres at the onset of prometaphase by approximately two to four minutes. The whole division cycle at 26°C. averages about thirty-five minutes, but in one case occupied only twenty-nine minutes. Thirty-eight minutes is the longest duration of one cycle as yet recorded. Data gathered in 1939 from a stock of *Pediculopsis* collected at Rochester, New York, are consistent in detail with these observations made on a collection of *Pediculopsis* from Long Island, 1941.

The spindle fibres which become visible at prometaphase and persist to telophase, appear as continuous streaks or striae—or perhaps delicate lamellae—from pole to pole. They appear dull gray, being less refringent than the peculiar material accompanying the chromosomes to the equatorial plate. In polar view of metaphase the spindle appears as a fairly regularly speckled structure due to the aggregate optical cross-sections of the "fibres." For the most part the fibres appear to pass, but not run to, the curious refringent materials located at the equatorial plate. It is clear, therefore, that the spindle elements are chiefly primary or continuous spindle fibres.

In testimony of the normality and health of the eggs studied, it should be pointed out that a blastomere whose spindle shows a pronounced fibrous structure not only completes its normal cleavage, but two or more directly descendent generations of blastomeres may likewise be followed through all stages of their mitoses in the same preparation. Step for step the process is the same; in all the spindle once formed is fibrous. In *Pediculopsis graminum* (Reut.), at least, there is visible evidence of the morphological reality of spindle fibres.

* The initial observations were made at Columbia University and the University of Rochester in the fall of 1938 and spring of 1939, respectively.

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[5] Cleveland, L. R., *Mem. Amer. Acad. Arts and Sci.*, **17**, 211 (1934), reports and illustrates fibres in living achromatic figures of certain Hypermastigina (Protozoa). The fibres of what he terms a "central-spindle" as well as those to the outer walls of the nu-

cleus opposite the chromosomes are very interesting, but of questionable significance. If, as Cleveland asserts (op. cit., also, *Science*, **81**, 598 (1935)), all of these fibres are merely astral rays, then it is not surprising that they are visible in the living organism. Moreover, the homology of such a bizarre achromatic apparatus with that of multicellular organisms is by no means clear.

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[9] The "refringent materials" in fixed preparations stain selectively, and thus may be shown not to be chromosomoids. They are not considered in my earlier account, for they showed only under certain fixation procedures. It was the attempt to ascertain whether these materials exist in the living egg that led to the discovery of the visibility of the spindle fibres.

GERM CELL MOVEMENTS AND SEX DIFFERENTIATION OF THE GONADS IN THE DROSOPHILA EMBRYO

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The development and differentiation of the gonads in wild type larvae and pupae of *Drosophila melanogaster* were studied by Kerkis[1] who found that the sex of the gonad is already determined in freshly hatched larvae. The size of the gonad and its relation to the adjacent fat body were the characteristics used to determine whether a gonad was female or male. Female gonads were considerably smaller in size than were male gonads and were embedded in the fat body while male gonads were only bordered by that tissue. The indifferent stage in gonad development, that is, one in which the sex of the gonad cannot be distinguished by a histologic examination, was therefore not found in the larva and it remained to be determined whether such a condition existed in an earlier developmental period. Preparations of embryos of known age have been examined and, as the present report will indicate, it has been found that the sex of the gonad is apparently determined as soon as the definitive gonads are formed. Thus, there appears to be no indifferent stage in the development of the gonad.

By the tenth hour after fertilization in embryos incubated at 25°C., the rather complicated and tortuous wanderings of the germ cell primordia have been completed. At that stage of development the primordia are in two groups, surrounded by a number of small mesentodermal cells and situated dorso-laterally in a position four segments from the posterior end of

the organism. The gonads will remain at this site during the remaining period of embryonic and post-embryonic development. The present paper contains brief descriptions of (*a*) the movements of the germ cells from the time of their origin until they are incorporated in the gonads and (*b*) the earliest feature of sex differentiation, gonad size, to become apparent.

Methods.—In *Drosophila melanogaster* the majority of larvae raised at 25°C. emerge from the embryonic envelopes during the twentieth hour of development. If in such a rapidly developing organism critical embryological studies are to be made, it is obviously essential that excellent material and carefully maintained conditions be employed. A highly vigorous and fertile Oregon-R strain was used that had been sustained in mass cultures for several years and had consistently given from 94 to 97% egg hatchability.[2] Mated females were permitted to lay eggs on the inside surface of ripened banana skins and the strips were then placed in an incubator regulated to function at 25 ± 0.3°C. Since Drosophila females may retain eggs in their uteri for varying lengths of time, it was necessary to use methods which would ensure that the embryos of any one batch were at almost the same level of development when fixed. The flies were first allowed to lay eggs for one hour. The experiment was continued only if many eggs were found to be deposited during this first period. In that case the banana strip containing these earlier eggs was discarded and a fresh strip introduced into the bottles. The second laying period did not exceed thirty minutes and the age of the embryos was calculated as from the midpoint of the laying period. Observations on living, dechorionated eggs and on groups of sectioned eggs and embryos indicate that the material collected in this manner was, on the whole, at similar stages of development.

Formation of the Gonads.—The first four hours of development, including the polynuclear origin of the germ cells, have been described in detail.[3,4] The synchronously dividing nuclei increase by powers of two and at the time when 256 are present they appear in the cortical layer of the egg, arriving in the posterior polar region somewhat earlier than in the other regions of the egg. A variable number of protuberances become manifest at the posterior pole and the nuclei which happen to be present in the granular polar region enter the outpocketings. These protuberances or buds become fully detached from the cytoplasmic region above and eventually, following the formation of the continuous layer of columnar blastodermic cells, are compressed into the area between the flat dorso-posterior blastodermic wall and the vitelline membrane. These small spherical cells which form an amicropylar polar cap are the pole cells or primordial germ cells and comprise the first obvious differentiation of the developing Drosophila egg. I have observed in living, dechorionated eggs that the polar protrusions rapidly become enlarged and distinct and within ten

minutes after they first appear their continuity with the ooplasm above is lost and they are fully detached in the perivitelline space. The germ cells remain massed at the caudal end of the egg for a period of seventy minutes.

Counts of the germ cell primordia which pass from the postero-dorsal perivitelline area into the interior of the egg have been given recently by Rabinowitz.[4] He notes that the average of 55 primordia (with a range in counts in different eggs of from 36–73) initially present decreases to an average of 31 germ cells (with a range from 15–44) which are, ultimately, to pass into the deepening amino-proctodeal invagination. Rabinowitz accounts for this decrease by describing an "inter-blastodermal migration" during which a number of free, detached pole cells pass between the elongate blastoderm nuclei and make their way toward the yolk. Counts made by the writer some time ago indicate that an average of 40 primordia enter the interior of the embryo and that more than fifty may be observed in certain preparations grouped at the posterior end of the diverticulum. These counts are of interest in light of the observations on gonad size which are to be described in the following section.

In living material, the cellular movements involved in the formation of the shallow amino-proctodeal concavity about the germ cells and the shifting of the invagination dorsally to a point approximately one-fifth of the distance from the caudal end occur within thirteen to seventeen minutes. The invagination deepens and the primordia, carried passively along in the concavity, disappear from sight as they move to the end of the lengthening proctodeal tube. In four- to six-hour-old embryos, the primitive germ cells may be seen loosely assembled in the diverticulum. No indication of the presence in the cytoplasm of the primordia of polar granules, the so-called "germ cell determinants," is evident at this time although Poulson,[5] who has made thus far the only comprehensive study of Drosophila organogenesis, reports that the germ cells are readily recognizable when in the invagination by the polar granules in their cytoplasm.

The germ cells remain quiescent at the end of the tube for two hours and sometime during the sixth hour of development a true migration occurs. The primordia pass through the amino-proctodeal wall into the body cavity. This movement, occurring in the interior of the embryo, cannot be seen in living material. How such movements occur has not been reported. From a study of preparations of embryos fixed with heated formol-alcohol-acetic acid mixture (70°C.) in which the contours of individual cells stand out strikingly, supplemented by preparations of embryos treated with cold fixative, the impression gathered is that the cells extend blunt pseudopodia and push their way singly through the gut wall. The primordia assume shapes which recall various ameboid types, and as they pass between the columnar cells of the gut they enlarge the spaces between the loosely aligned cells distorting the appearance of the gut com-

ponents. The passage through the gut wall must occur within a period of about an hour for in embryos which are seven to eight hours old the migration is completed. Various steps in the migration can be readily traced in different preparations. Not all of the primordia, however, which were carried passively into the proctodeal tube in the early embryo penetrate into the body cavity. It is difficult to ascribe a reason for this since, presumably, all of the germ cells contain identical nuclear complexes and since differences in appearance or size of the various primordia are not discernible to the observer. Many of the germ cells remain behind in the gut and are never incorporated in the definitive gonads. In older embryos, those from thirteen hours and on, cells which resemble the primordia are occasionally noted in the gut. It may be inferred that these are the survivors of those germ cells which remained behind during the migration which occurred hours earlier.

The wanderings of the germ cells are, even yet, incomplete. From their position in the body cavity between the blind end of the proctodeal tube and the huge yolk aggregate, the primordia have to move or be moved to their final latero-dorsal position four segments from the caudal end of the embryo. This final step is exceedingly difficult to follow, but it occurs during the posterior retreat of the proctodeum from its anterior position. With the onset of metamerism, the proctodeal tube, opening on the dorsal surface and extending posteriorly, begins to move caudally. Eventually, the proctodeal opening reaches the back end of the embryo, thus reversing the movements initiated hours earlier at the time of the dorsal shifting of the germ cells.[5] The mesentodermal cell cluster associated with the tip of the proctodeal tube becomes cleft and as the strands move back and then turn ventrally to form a portion of the mid-gut wall they appear to shunt the two groups of germ cells to their permanent sites. Here the primordia are surrounded by a number of small mesentodermal cells from which material the future gonadal sheath is differentiated. In fourteen-hour-old embryos the sheath is quite distinct, comprising a circlet of minute cells about each cluster of germ cell primordia. The number of cells in the sheath has been determined and found to vary from 26 to 37. Care must be taken in making these computations to distinguish the sheath cells as carefully as possible from the neighboring mesentodermal cells, some of which are differentiating into fat body.

Sex of the Gonads.—Sex differentiation of the gonad has already occurred in larvae newly emerged from the embryonic cases.[1] The size difference between the gonads is quite striking and in six- to ten-hour-old larvae the male gonads are developed far enough to be visible through the body wall, enabling one to separate the sexes in the larvae without any recourse to dissection and measurement. The female gonads are minute and Kerkis remarks that "the young female gonads are so small that it is difficult to

measure them as accurately as the male gonads or the older female gonads." To establish whether an indifferent stage in gonad development exists, embryos have to be killed and sectioned since it is not possible to dissect out the gonads in embryos and measure their dimensions. Poulson[5] has noted that the gonads in some "late embryos" were different in size. This observation we have confirmed and have, furthermore, made counts of the number of primordia present in the gonads of embryos which were at different levels of development. Germ cell counts in a sample of 110 gonads in 72 embryos were obtained and the following comments can be made.

1. A size difference is observed as soon as the primordia have arrived at their permanent location. This difference is apparent in embryos which have incubated for ten hours and is the earliest discernible feature of sex differentiation. This disparity in volume of the gonads can be traced from this time on until the young larva hatches. From these and from Kerkis' observations it can be concluded that no indifferent stage in the development of the gonad exists and that the sex of the gonad is established at the time the germ cell primordia have completed their wanderings and have become incorporated in the gonads.

2. Variations in volume of the gonads results from the number of seemingly identical primordia enclosed in the sheath rather than any difference in size of the primordia. The numbers of germ cells in the early gonads fall, roughly, into two groups, those containing 5 to 7 cells per gonad (there is one instance of 4 primordia in a gonad) and those containing 9 to 13 germ cells. The numbers of primordia in the two members of any pair of gonads are virtually the same. One may note, for example, 6 primordia in one gonad and 6 or 7 in the other, but not 6 in one gonad and 13 in the other member of the pair.

3. When one considers that approximately 40 germ cells, and sometimes more, enter the interior of the body through the amino-proctodeal invagination but that gonads are formed which contain 5, 6, or 7 of the primordia, it is obvious that large numbers of the potential germ cells have been "lost" during the course of the movements undergone by the primordia. As noted earlier, many primordia are unable to migrate from the end of the the proctodeal tube into the body cavity, while some in the body cavity appear to be trapped in the large yolk aggregate which is later included in the mid-gut.

4. Following the initiation of the complex movements in the early embryo, no mitoses are ever noticed among the primordia until the embryo has incubated for sixteen hours, while in the proctodeal tube and in the body cavity, the germ cells have never been observed dividing, and during the first few hours after their inclusion in the gonads the primordia are still seemingly quiescent. Between the sixteenth hour of development and hatching, however, one, and possibly two, mitoses occur. Gonads are now

observed which contain 8, 10 and 12 primordia while others have as many as 36 and 38 primordia. These figures are interesting since Kerkis showed that the ratio of the volumes of the larger (male) gonads to the smaller (female) gonads in newly emerged larvae was of the order of $3^1/_2$ to 1. It can also be understood why Kerkis had difficulty in determining the dimensions of female gonads in young larvae for so very few cells are contained in a female gonad in a late embryo. Even should one or two mitotic divisions occur during the first larval hours such gonads would still be extremely small and most difficult to measure.

Summary.—The germ cells have been traced from the time of their origin to their inclusion in the primitive gonads. Many germ cells which appear in the early embryo never reach the gonads. Size differences in the gonads have been observed and it is concluded that no indifferent stage in the development of the gonad exists.

* Fellow, John Simon Guggenheim Memorial Foundation.

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ON THE INDEPENDENCE OF LINE INTEGRALS ON THE PATH

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The investigations of Menger[1] and Fubini[2] have renewed the interest in the question of the independence of the line integral on the path. I shall offer here a necessary and sufficient condition that is purely local and contains the classic conditions for Cauchy's theorem as well as those for Green's formula. For the sake of simplicity we restrict ourselves to two variables, x, y.

A differential dA shall be any function of the four variables x, y, dx, dy, and the $\int_C dA$ along a curve C shall be defined the obvious way (if it exists). We shall assume that the integral changes the sign if we invert the direction of the curve C. (It would be easy to give conditions for that, too.)

Definition.—The differential dA is called regular at a point P if for any sequence Δ_n of similar triangles with areas a_n of limit 0 we have

$$\lim_{n=\infty} \frac{1}{a_n} \int_{\Delta_n} dA = 0$$

No uniformity in the limit as to the shape of the triangles is requested.

THEOREM 1. *In a simply connected domain we have* $\int_C dA = 0$ *for any triangle of the domain if and only if* dA *is regular at every point of the domain.*

Proof.—The condition is obviously necessary. For sufficiency we follow the well-known proof of Cauchy's theorem. A sequence Δ_n of triangles similar to Δ is constructed, each half the size of the preceding and contained in it, so that

$$\left| \int_\Delta dA \right| \leqslant 4^n \left| \int_{\Delta_n} dA \right|.$$

Let a be the area of Δ and therefore $a/4^n$ the area of Δ_n. If P is the common point of all Δ_n we have for sufficiently large n that $\left| \int_{\Delta_n} dA \right| \leqslant \frac{a}{4^n} \epsilon$.

This proves the theorem.

It contains as special case:

THEOREM 2. *Let* $P(x, y)$ *and* $Q(x, y)$ *have differentials in the sense of Stolz at the point* x_0, y_0 *and in addition to that* $\partial P/\partial y = \partial Q/\partial x$ *at* x_0, y_0. *Then* $Pdx + Qdy$ *is regular at* x_0, y_0.

Proof.—Our assumptions lead to an approximation of P and Q by linear functions such that the coefficient of y in P equals that of x in Q. In a sufficiently close neighborhood of x_0, y_0 the error will be less than $\epsilon \cdot \delta$ where $\epsilon > 0$ is given in advance and δ is the distance of x, y and x_0, y_0. The integral along Δ_n over the linear function yields zero, the integral over the error is of magnitude $\epsilon \cdot a_n$.

THEOREM 3. *If* $\partial P/\partial y$ *and* $\partial Q/\partial x$ *exist in a certain neighborhood of* x_0, y_0, *are continuous at that point and equal in* x_0, y_0, *then our differential is again regular.*

Proof.—This time we approximate $P(x, y) = P(x, y_0) + (y - y_0) \times (\partial P/\partial y)\ (x, y_1)$ by a function of the form $\varphi(x) + a(y - y_0)$ where $\varphi(x)$ depends on x alone and a is a constant, and $Q(x, y)$ by $\psi(y) + a(x - x_0)$. The error is of the same order of magnitude as before. Since $\int_{\Delta_n} \varphi(x)dx = \int_{\Delta_n} \psi(y)dy = 0$ and $\int y\,dx + x\,dy = 0$ we have again to consider merely the error term.

[1] Menger, Karl, "On Cauchy's Integral Theorem in the Real Plane," *Proc. Nat. Acad. Sci.*, **25**, 621–625 (1939).

[2] Fubini, Guido, "On Cauchy's Integral Theorem and on the Law of the Mean for Non-derivable Functions," *Ibid.*, **26**, 199–204 (1940).